

Sperm Competition and the Evolution of Millipede Genitalia

Mandy BARNETT * & Steven R. TELFORD **

* Department of Zoology, University of Cape Town, Rondebosch 7700, South Africa

** Department of Zoology, University of Pretoria, Pretoria 0002, South Africa

ABSTRACT

Natural selection has presumably shaped much of genital morphology for the efficient transfer of sperm, but does not account for the evolution of seemingly bizarre male genitalic appendages. Gonopods of several species of spirostreptid millipedes were examined using light and scanning electron microscopy, and the sequence of events representative of their movement within the spermathecae demonstrated through the dissection of freeze-dried copula pairs and simulations using scale models. Gonopods bear devices that may function in sperm displacement, including flagellae with ridges and overlapping plates, scoops and regions of pitted spines. These are orientated correctly so as to facilitate sperm removal and are accommodated within the spermathecae of the females. This morphological evidence, coupled with spirostreptid physiology and behaviour, indicates that sperm competition may have played a major role in shaping gonopod morphology.

RÉSUMÉ

Transfert compétitif du sperme et évolution des genitalia des diplopodes.

La sélection naturelle a vraisemblablement beaucoup contribué à conformer la morphologie des genitalia pour un transfert efficace du sperme, mais elle n'explique pas l'évolution morphologique en apparence bizarre des appendices génitaux des mâles. Les gonopodes de plusieurs espèces de diplopodes spirostreptides ont été examinés en microscopie optique et en microscopie électronique à balayage et la séquence des événements traduisant le mouvement des gonopodes à l'intérieur de la spermathèque a été mise en évidence par la dissection des pièces copulatrices et par des simulations à partir de modélisations. Les gonopodes portent des dispositifs variés qui interviennent dans le déplacement du sperme, incluant flagelles avec arêtes, lames se recouvrant, concavités et zones recouvertes d'épines enfoncées. Ils sont orientés de manière à faciliter la réception du sperme et s'accordent à la morphologie de la spermathèque des femelles. Cette évidence morphologique, couplée avec la physiologie et le comportement, indique que la compétition pour le transfert du sperme a pu jouer un rôle majeur dans la conformation morphologique du gonopode.

INTRODUCTION

Gonopods are taxonomic characters of primary importance in many millipedes (HOPKIN & READ, 1992) but, curiously, the selective processes responsible for the evolution of these complex structures have not been considered. Selection for effective sperm transfer presumably accounts for much of gonopod morphology, but, as in many taxa with complex genitalia (EBERHARD, 1985), does not fully explain their dramatic diversity.

The most likely explanation for this genital complexity is sexual selection, conceived by DARWIN (1871). Sexual selection is believed to operate through intrasexual (usually male-male) combat and intersexual (usually female) choice. In the context of intraspecific competition, sexual selection is believed to favour devices and behaviours of males that would prevent interference from other males before and during copulation (THORNHILL & ALCOCK, 1983). A significant new dimension to sexual selection theory is the concept of competition between the ejaculates of two or more different males for the fertilisation of ova (PARKER, 1970). Sperm competition occurs between the time of insemination and fertilisation. Mechanisms of sperm competition include the stratification, removal and dilution of ejaculates (BIRKHEAD & HUNTER, 1990). Because sperm competition is a powerful selective force in the evolution of reproductive behaviour and genital morphology (PARKER, 1970; SMITH, 1984; BIRKHEAD, 1989) it may simultaneously favour the evolution of devices that enhance an individual's ability to displace, replace or dilute a rival gametes, and behaviours that resist preemption of ejaculates (PARKER, 1970; WAAGE, 1984, 1986a; and see SMITH, 1984).

The behaviour and genital morphology of spirostreptid millipedes can be interpreted in the context of sperm competition. All the provisos for the evolution of sperm competition are fulfilled in millipede mating systems: they are polygynandrous, store sperm and fertilisation is delayed (TELFORD & DANGERFIELD, 1993a,b, c). Males protect their reproductive investment in females by prolonging the duration of copulation; a behaviour that is best interpreted as a form of mate guarding (TELFORD & DANGERFIELD, 1991, 1993c; BARNETT & TELFORD, 1994).

Here we focus on genitalic functional morphology and argue that gonopods are adaptive devices designed to displace (via stratification or removal) rival ejaculates. In support of this hypothesis, we present evidence to demonstrate that the gonopods reach the distal ends of the spermathecae, bear the necessary devices with which to displace sperm and, in some species, move within the spermathecae to effect sperm displacement.

MILLIPEDE GONOPODS

Millipede gonopods comprise three components: the sternite, the coxite and the telopodite, the latter of which contains the sperm canal. The gonopods are normally drawn into the body of the male so that only the distal ends of the coxites are visible. During copulation they are protruded and sperm are transferred from the penes to the coxite from where they are released into and stored in the spermathecae of the vulvae of the female (BARNES, 1986; KRABBE, 1982; BLOWER, 1985).

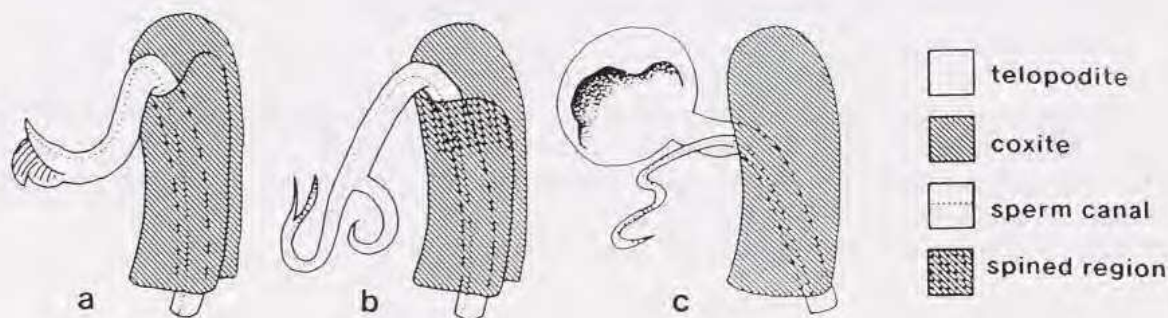


FIG. 1. — General plan of the LHS gonopod of (a) Harpagophoridae, (b) Spirostreptidae and the RHS gonopod of (c) Odontopygidae.

During copulation, the telopodite is retracted and released, causing it to perform a sequence of twists and turns that depend on the configuration of the telopodite arm and its association with the coxite. The general association of these components is family specific (Fig. 1).

In the Harpagophoridae and the Spirostreptidae, the telopodite is held within the gonocoel, a fold formed by the coxite. It originates at the base of the gonocoel and rises to its opening where it bends outwards, traversing the top of the lateral margin of the coxite. In the Harpagophoridae the telopodite typically ends in a rigid comb-like structure (ATTEMS, 1928, 1937). In the Spirostreptidae the telopodite varies from a single arm to one which bifurcates medially (ATTEMS, 1928, 1937). In addition, the Spirostreptidae typically bear a region of spines on the distal oral coxite (ATTEMS, 1928, 1937). These vary in form from stout to hair-like spines that may or may not be situated in pits.

The Odontopygidae have dramatically different gonopods. The telopodite originates at the base of the coxite but, because the coxite has no gonocoel, is not held within it. Instead it passes behind the coxite and bends inwards. Telopodites are proportionally larger than those of the Harpagophoridae and the Spirostreptidae and also bifurcate. The sperm canal is held within the whip-like arm.

GONOPODS AS DISPLACEMENT DEVICES

In order to actuate displacement, the gonopods need to bear morphological devices with which to manipulate rival sperm. In species shown to displace sperm (MC VEY & SMITTLE, 1984; SIVA-JOTHY, 1984, 1987; WAAGE, 1986a, b; MICHIELS & DHONT, 1988; RUBENSTEIN, 1989; MILLER, 1991; VON HELVERSON &

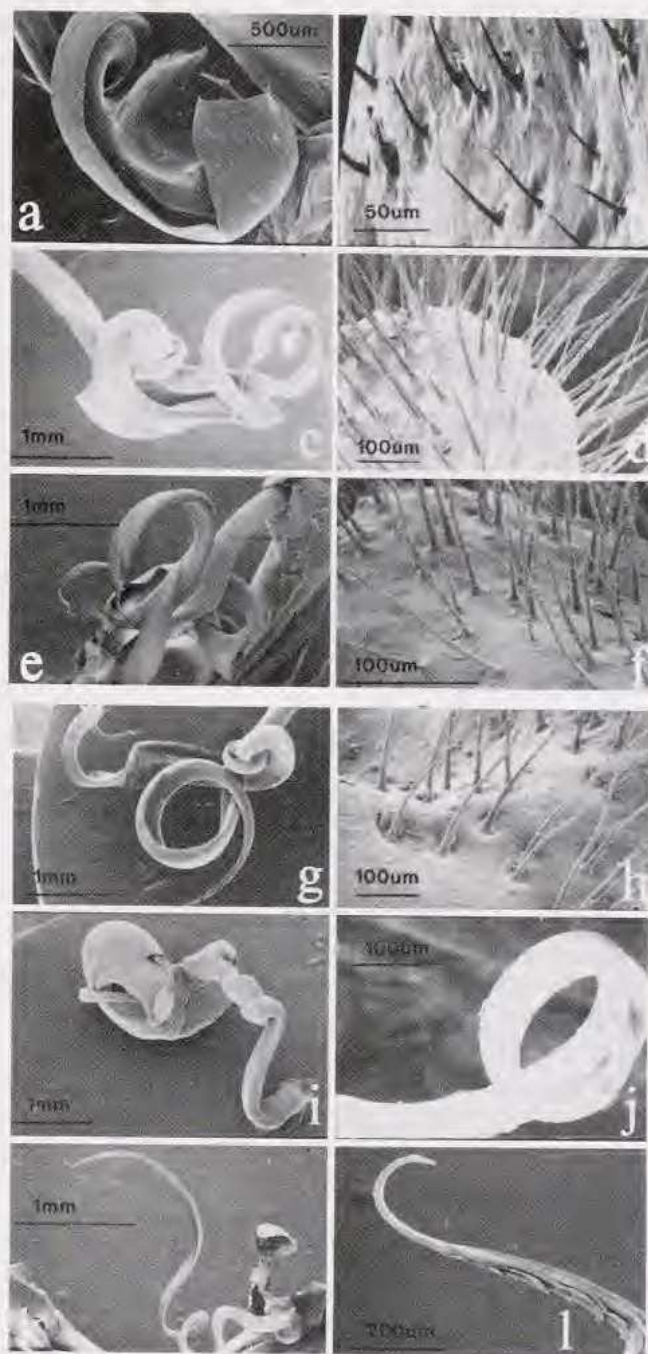


FIG. 2. — Scanning electron micrographs of gonopod features. *Orthoporus pyrocephalus* distal telopodite scoop (a) and region of spines (b); *Alloporus* sp. telopodite end (c) and spines (d); *Alloporus uncinatus* telopodite end showing medial scoop (e) and spines (f); *Doratogonus* sp. telopodite end (g) and spines (h); *Chaleponcus* sp. telopodite scoop (i) and distal end of sperm-canal bearing arm (j); *Chaleponcus limbatus* sperm-canal bearing arm (k) and overlapping plates at its distal end (l).

VON HELVERSON, 1991; GAGE, 1992) the morphological devices that have evolved to facilitate displacement include scoops (WAAGE, 1982), spines (WAAGE, 1986a, b) and flagellae with overlapping barbs (WAAGE, 1984).

Scoop-like structures occur on the telopodites of several species of millipede belonging to the families Odontopygidae and Spirostreptidae. These vary in form and in their position on the telopodite. In *Orthoporoides pyrocephalus*, L. Koch the telopodite terminates in a spade-like structure (Fig. 2a). In *Chaleponcus* sp. the telopodite bifurcates, and one arm (the one not bearing the sperm canal) ends in a large rounded scoop (Fig. 2i). In addition, the sperm-canal bearing arm of the telopodite bears a series of ridges at its distal end (Fig. 2j). The telopodite of *Chaleponcus limbatus* also bifurcates with the sperm canal arm bearing a series of backwardly overlapping plates (Fig. 2k, l), and the other arm terminating in a less rounded scoop-like structure.

In *Alloporus* spp. and *Doratogonus* sp., where the telopodite also bifurcates, a trowel-like scoop occurs half way up the sperm canal bearing arm (Fig. 2c, e, g). The telopodites of species belonging to the family Harpagophoridae are more robust and terminate in rigid comb-like structures.

Interestingly, spines are found in some species belonging to the family Spirostreptidae but not in the other two families of Spirostreptid millipedes. The distal end of the oral region of the coxite is the only place on the gonopod where spines occur. These spines vary in form from stout pitted spines to long hair-like spines that are pitted in some species (Fig. 2b, h) and not in others (Fig. 2d, f).

COMPATIBILITY OF GONOPOD SIZE AND SPERMATHECAL SHAPE

To manipulate the sperm of rivals, a male's genitalia need to be able to access the areas of the female in which sperm are stored (PARKER, 1970; WALKER, 1980; KNOWLTON & GREENWELL, 1984; WAAGE, 1986a). In insects, spermathecae range from relatively simple structures to complex convoluted organs, the latter of which may restrict access of the male genitalia to the site of sperm storage (EBERHARD, 1985). If sperm competition occurs via displacement, then spermathecal shape and size show strong correspondence (e.g. damselflies: WAAGE, 1984, 1986a; dragonflies: WAAGE, 1986a; MILLER, 1991; and see WALKER, 1980; EBERHARD, 1985) to the size and shape of male genitalia (WAAGE, 1984, 1986a). This may prove to be a generalisation that holds true for all invertebrates that displace sperm.

In millipedes, female gonopores open into paired vulvae (BLOWER, 1985; HOPKIN & READ, 1992). These are opaque structures containing roughly oval chitinous structures which form the inner chambers of the spermathecae. The spermathecae open distally into oviducts that join to form a common oviduct running posteriorly to the ovaries (BARNETT, TELFORD & DE VILLIERS, 1993). Millipede spermathecae are relatively simple structures that are species specific in both shape and size (Fig. 3).

To actuate effective manipulation (and placement) of sperm, selection should favour the evolution of structures that can reach the areas of the spermathecae where the sperm are stored. In millipedes the chitinous inner chamber of the spermatheca appears to be the main site of sperm storage (unpublished data). For each species examined, the distal ends of the telopodites of males can be accommodated within the spermathecae and can easily reach their distal ends. Thus, the manipulation of sperm held within these regions of the female reproductive tract is possible.

Female sperm storage organs can be very complex structures with highly sophisticated muscular control (e.g. VILLAVASCO, 1975). Thus, females may be able to exert some control over fertilisation events resulting in selection acting on males to overcome this control. This conflict of interest can generate an escalating evolutionary spiral, or arms race (*sensu* DAWKINS & KREBS, 1979) between the sexes to gain control over copulatory events. The outcome of this process of co-evolution would be concomitant genitalic adaptation and perhaps structural complexity. The latter is true for millipede gonopods but not spermathecae.

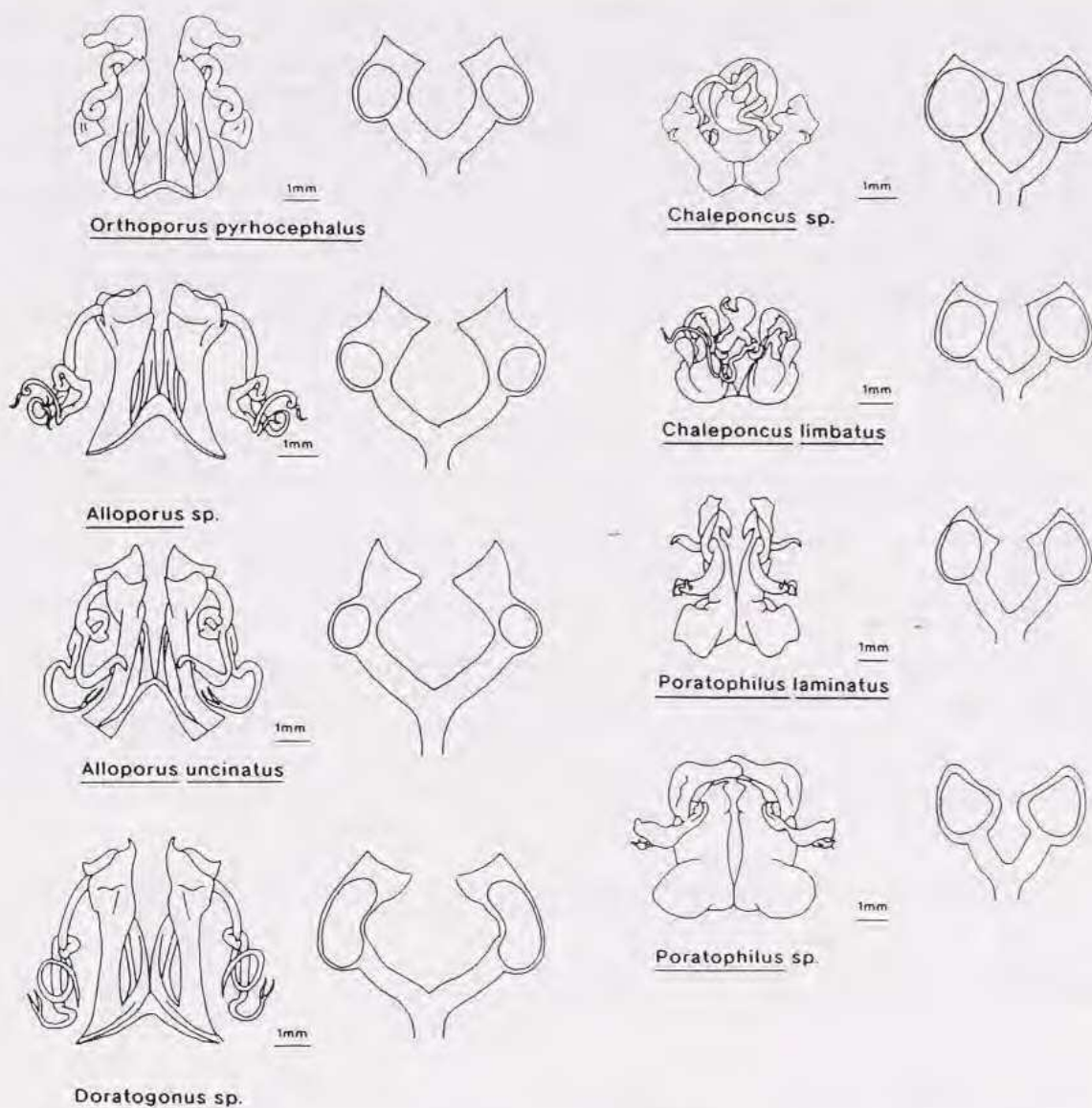


FIG. 3. — Diagrammatic representations of corresponding gonopods and spermathecae. The oval shapes within the spermathecae represent the chitinous inner chambers. (*Orthoporus* = *Orthoporoides* in the text; *Poratophilus* = *Zinophora* in the text)

Interestingly, the size of the genitalia is not related to the body sizes of the animals. *Chaleponcus* sp. is one of the smallest species (mean mass(g) = 2.49, SD = 0.4, n = 45) and its spermathecae are as large as those of *Alloporus uncinatus* (mean mass(g) = 9.31, SD = 5.99, n = 295). Spermathecal size and shape in *Chaleponcus* sp. corresponds precisely with the large scooped distal ends of the telopodites of the gonopods of conspecific males. It should also be noted that present descriptions of spermathecal shape are based on external topography; internal shape may be different. Also, gonopods and/or female musculature may expand and alter the shape of spermathecae during copulation (see VILLAVASCO, 1975; SIVA-JOTHY, 1987; WALKER, 1980; MILLER, 1987, 1991).

GONOPOD ORIENTATION AND MOVEMENT WITHIN THE SPERMATHECAE

Movement of the gonopods is effected by retracting the proximal end of the telopodite and is determined by both the point of emergence of the telopodite from the coxite and the shape of the telopodite arm. Retraction-release sequences have been reconstructed for two of the species examined here, and illustrate that describing the structure of the distal end of the telopodite is not sufficient to fully explain its functional morphology. The shape of the telopodite arm, and particularly the number and tightness of spirals that it describes are critical in determining its movement path within the spermatheca and hence its sperm displacement action. For example, in *Orthopoides pyrocephalus*, the telopodite emerges at the distal end of the coxite. When it is retracted, it traverses the bridge formed by the margin at the fold of the coxite. The scoop at its distal end twists within the spermathecae and is then brushed against the spined region on the distal coxite. This movement may be a mechanism whereby sperm could be removed from the spermatheca prior to insemination (Fig. 4).



FIG. 4. — Telopodite retraction-release cycle for the RHS gonopod of *Orthopoides pyrocephalus*. Proximal retraction of the gonopod causes the distal end to rise (2); twist forward (3); twist back (4); twist forward (5) and then flip round to make contact with the spined region of the coxite. Release of the telopodite results in the scoop brushing downwards against the spines. Source: Modified from BARNETT, TELFORD & DE VILLIERS 1993.

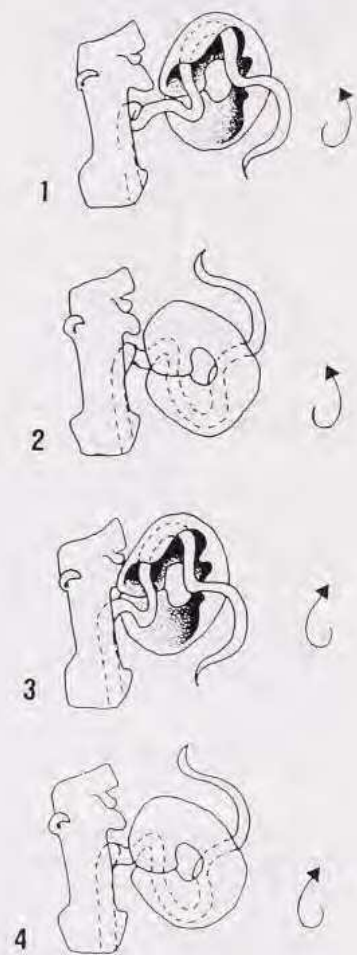


FIG. 5. — Telopodite retraction-release cycle of *Chaleponcus* sp. Retraction causes the scoop to twist about its own axis, flipping forward (2); forward again (3); then backwards (4) and backwards again (1).

In contrast, the telopodite of *Chaleponcus* sp. emerges from the posterior base of the coxite and retraction yields a rotation of the scoop about its own axis. There are no spines against which the scoop is brushed, and it is predicted that the scoop functions to reposition or mix sperm within the spermathecae but not to remove it (Fig. 5).

This comparison serves to illustrate how two apparently similar structures can have different functions due to the shape of the telopodite arm and its resultant plane of movement. Thus, the evolution of sperm displacing devices in millipedes will not only be linked to the actual displacing structures, namely the distal ends of the telopodites, but to the gonopods as a whole because the mechanism of movement is dependent on the coxite, the shape of the telopodite arm, and associated structural modifications. This is in contrast to the damselflies in which simple horizontal movements during copulation make it possible to predict the mechanism of sperm competition from the morphology of the terminal region of the penis (WAAGE, 1984, 1986a).

CONCLUSIONS

The evolution of complex genital morphology in millipedes can be explained and understood in the context of sperm competition. Gonopods display the design features necessary for efficient sperm displacement and their complexity is probably a product of sexual selection via sperm competition. While sperm competition implies a focus on intermale competition, the evolutionary perspective of females is also critical to understanding genitalic evolution (KNOWLTON & GREENWELL, 1984). The spermathecae provide the arena for competitive interactions and females may be capable of dictating the outcome of the competition (WALKER, 1980; EBERHARD, 1985).

The relatively simple spermathecal structures of female millipedes contrast with male gonopod complexity. This suggests that the manipulative capabilities of the gonopods dictate the intensity of sperm competition and resultant patterns of sperm precedence.

Structural modifications of the terminal region of the telopodite and the shape of the telopodite arm together may provide an accurate prediction of gonopod functional morphology (see Table 1).

TABLE 1. — Between species comparisons of gonopod functional morphology, predicted mechanisms of sperm competition and sperm precedence patterns.

| FAMILY | SPECIES | GONOPOD FEATURES | | DISPLACEMENT MECHANISM | PRECEDENCE PATTERN |
|-----------------|------------------------|-----------------------------|------------------|---------------------------|-----------------------|
| | | TELOPODITE | COXITE | | |
| SPIROSTREPTIDAE | | | | | |
| | <i>O. pyrocephalus</i> | distal scoop | stout spines | removal | last male |
| | <i>A. uncinatus</i> | medial scoop | hair-like spines | stratification | last male |
| | <i>Alloporus</i> sp. | medial scoop | hair-like spines | stratification | last male |
| | <i>Doratogonus</i> sp. | medial scoop | hair-like spines | stratification | last male |
| ODONTOPYGIDAE | | | | | |
| | <i>C. limbatus</i> | distal scoop & flagellum | -- | removal | last male |
| | <i>Chaleponcus</i> sp. | distal scoop & flagellum | -- | stratification | first male |
| HARPAGOPHORIDAE | | | | | |
| | <i>Z. laminata</i> | distal comb | -- | removal | last male |
| | <i>Zinophora</i> sp. | distal comb | -- | removal | last male |

Where displacement of rival ejaculates occurs then last-male sperm precedence is the expected outcome of a multiple mating sequence (see WAAGE, 1986a; MILLER, 1991). Our data

predict last-male precedence in all but one species (*Chaleponcus* sp., Table 1). We have shown that sperm mixing or first-male precedence is likely to occur in this species (unpublished data). This is because the scoop-like terminal region of the telopodite redistributes rather than removes rival ejaculates; a consequence of the shape of the telopodite arm. These results suggest a cautious approach to ascribing a precise function to a structure without a complete understanding of its mode of action.

Descriptive studies of genital morphology are an essential first step towards understanding the precise function of these complex structures. Knowledge of the mode of action of the gonopods allows *a priori* predictions to be made about mechanisms of sperm displacement and patterns of sperm precedence. This is an essential basis for beginning an iterative series of experiments and manipulations designed to quantify mechanisms of sperm competition in millipedes. Although numerous studies have quantified patterns of sperm precedence (see SMITH, 1984), few have attempted the more challenging task of unravelling the underlying mechanisms.

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